To: Mount, Dave[Mount.Dave@epa.gov]; Monson, Phil (MPCA)[phil.monson@state.mn.us]; Hoff, Dale[Hoff.Dale@epa.gov]
Cc: Swain, Ed (MPCA)[edward.swain@state.mn.us]; Engelking, Pat (MPCA)[pat.engelking@state.mn.us]; Kessler, Katrina (MPCA)[katrina.kessler@state.mn.us]; Tomasek, Mark (MPCA)[mark.tomasek@state.mn.us]
From: Erickson, Russell
Sent: Thur 5/16/2013 9:53:41 PM
Subject: RE: Wild rice study statistical questions

If I understand right, the issue here is not whether two seedlings in the same tube would be independent samples – they would not be.

Rather, the issue is whether two seedlings per tube provide an experimental unit with lower variability than a single seedling per tube (like using multiple fish in a tank, even though the tank is treated as a replicate) and whether this lower variability has advantages in the experimental design and analysis. And also whether the merits of using one or two seedlings are different depending on whether hypothesis testing or regression analyses are done.

I don't know the tradeoffs here – will using two plants per tube mean the total number of experimental units (tubes) is lower? In the one extreme of being able to keep the number of tubes constant and using twice as many, then the issue is simply whether using two plants per tube is more informative/less variable than a single plant, in which case two plants should be used regardless of the design and analysis method. In the other extreme of keeping the total number of plants constant (i.e. two plants per tube mean there will be half the total number of tubes), I would say it shouldn't be done, again regardless of the analysis method, because the lower n is likely more important than any lower variance among tubes. For situations between these extremes, the tradeoffs need some knowledge of how measurement variance depends on the number of plants.

Regarding regression analysis vs hypothesis testing, for the same number of experimental units, the issue is simply how to balance replicates versus treatment levels. Regression analysis generally needs more treatment levels and fewer replicates per level, but in this case there could still be a fair number of replicates and there is no need to necessarily make these replicates more "robust" if the tradeoff is having even fewer replicates. This all gets down to exactly what decisions the information in the experiment needs to support and really requires more information/discussion amongst us, but my initial reaction here is that the two plants per tube is likely not worthwhile.

Russell Erickson

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From: Mount, Dave
Sent: Thursday, May 16, 2013 2:55 PM
To: Monson, Phil (MPCA); Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: RE: Wild rice study statistical questions

Ah, got it.

My first concern would be whether two seedlings growing in the same chamber could be considered independent samples for hypothesis testing purposes. My guess is not, though there may be analysis techniques that can be used to deal with this.

My memory is that the previous experiments showed pretty well defined response curves, so I'm not sure whether too few exposure concentrations would be an issue. If the goal were to define point estimates for very low levels of effect (e.g., EC10 or less) then having greater data density in that concentration range would be important, but for EC20 or EC50 values, my memory is that previous experiments seemed to produce data sufficient to define those curves reasonably well.

Again, you should get Russ' input to make sure I'm not off base.

Dave

From: Monson, Phil (MPCA) [mailto:phil.monson@state.mn.us]
Sent: Thursday, May 16, 2013 2:48 PM
To: Mount, Dave; Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: RE: Wild rice study statistical questions

As I understand – at least in general principles, increasing numbers replicates (and I know this is different than number of test organisms) serves to provide for better hypothesis tests while increasing the number of treatment levels in a test serves to better fit a regression-based point estimate. With that said, increasing, if we can, the number of plants (to two plants) in each jar would/might help to maintain statistical robustness if we choose to run more treatment levels (for needs for regression) and in turn reduce the total number of replicates per treatment to deal with the growth chamber space that we have… Phil

From: Mount, Dave [mailto:Mount.Dave@epa.gov]
Sent: Thursday, May 16, 2013 2:05 PM
To: Monson, Phil (MPCA); Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: RE: Wild rice study statistical questions

Hi Phil—

I may be forgetting something important from those previous discussions, but it's not clear to me why a replication level that provides sufficient power for hypothesis testing would be insufficient for regression. In general, regression approaches tend to be less demanding of replication rather than more. But maybe I'm missing something.

Russ knows way more about it than I, I suspect he'll have something to add.

Dave

From: Monson, Phil (MPCA) [mailto:phil.monson@state.mn.us]
Sent: Thursday, May 16, 2013 12:37 PM
To: Mount, Dave; Erickson, Russell; Hoff, Dale
Cc: Swain, Ed (MPCA); Engelking, Pat (MPCA); Kessler, Katrina (MPCA); Tomasek, Mark (MPCA)
Subject: Wild rice study statistical questions

Hi you guys,

I wanted to give you brief update on our work with developing methods for testing wild rice in the lab.

Sulfate (aerobic) exposures

1. We think we've nailed down pH control (went with a PIPES buffer) and are able to hold it to 6.9 $\pm$ 0.2 pH units.

2. Are in the process of completing another set of range-finder tests using two methods

a. Seeds through germination – 50 seeds per test jar with 3 replicate jars per trt.

b. Emerging seedlings (1-2 day post germination) – 20 tubes per trt with one plant per tube.

Sulfide (anaerobic) exposures

1. Control of pH as described above with still some work needed at higher SO2 (100 micromole)

2. Will perform range finder tests within next month (tent.)

Perhaps the biggest issue will be the best approach for statistical analysis. I think we're in agreement that in order to best describe a dose-response relationship, regression analysis to establish a point estimate will be most favorable. I and Ed are a bit concerned that using only one plant per exposure chamber (tube) may limit the utility of performing a regression. Power test on these tube exposures (I think we shared this information with you at some point) indicated that around 20 tubes should be appropriate replication. That said, I'm toying with the idea to get some larger flasks (double the volume probably around 140 mL) so that we can use two germinating seeds per tube. I'd like your thoughts on this if you have time. In addition, discussion of an appropriate statistical procedure will be a topic at an upcoming (next week) phone conference with the "Technical" Advisory group (sub-group of the larger advisory committee). Perhaps the main point in that will be using regression vs. hypothesis testing as the preferred method and I would like to vet some options to the group for a preferred statistical approach.

Any thoughts you can share would be greatly appreciated.

Thanks!

Phil

Philip Monson

Research Scientist

Minnesota Pollution Control Agency

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